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# LncRNA MALAT1 Promotes STAT3-Mediated Endothelial Inflammation by Counteracting the Function of miR-590

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**Keywords**
Inflammation · lncRNA · MALAT1 · miR-590 · STAT3 signaling

**Abstract**
The excessive production of inflammatory mediators by vascular endothelial cells (ECs) greatly contributes to the development of atherosclerosis. In this study, we explored the potential effect of lncRNA MALAT1 on endothelial inflammation. First, the EC inflammation model was constructed by treating human umbilical vein ECs (HUVECs) and human coronary artery ECs (HCAECs) with oxidized low-density lipoprotein (ox-LDL), which confirmed the role of MALAT1 in the inflammatory activity. Then MALAT1 was overexpressed in HUVECs and HCAECs, and the levels of inflammatory mediators and nitric oxide (NO) were examined by Western blotting, ELISA, and NO detection assay. The migration ability was confirmed by wound healing assay. The interactions among MALAT1, miR-590, and STAT3 were predicted by bioinformatics analysis and verified by qRT-PCR, Western blotting, or dual-luciferase reporter assay. MALAT1 was upregulated in ECs treated with ox-LDL, and knockdown of MALAT1 significantly inhibited ox-LDL-induced inflammation. MALAT1 overexpression potentiated the inflammatory activities of ECs, including enhanced production of inflammatory cytokines (IL-6, IL-8, and TNF-α) and adhesion molecules (VCAM1 and ICAM1), and decreased NO level and cell migratory ability. Mechanistically, MALAT1 could directly downregulate miR-590, and miR-590 could bind to the 3′-UTR of STAT3 to repress its expression. Additionally, overexpression of MALAT1-mediated inflammation was largely abrogated by the concomitant overexpression of miR-590. miR-590 knockdown activated the inflammatory response, which was reversed by STAT3 inhibition. Thus, MALAT1 serves as a proinflammatory lncRNA in ECs through regulating the miR-590/STAT3 axis, suggesting that MALAT1 may be a promising therapeutic target during the treatment of atherosclerosis.


Atherosclerosis is one of the leading causes for multiple types of cardiovascular disorders such as coronary disease, cerebral infarction, and myocardial infarction [Nicorescu et al., 2019]. From an etiological perspective, atherosclerosis can be seen as inappropriate inflammatory responses mediated by abnormal activation of immune cells, such as monocytes, macrophages, or T lymphocytes inside the artery wall. Apart from immune cells, the function of vascular endothelial cells (ECs) also has a

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profound impact on the development of atherosclerosis [Krupinski et al., 2008; Geovanini and Libby, 2018]. Under physiological conditions, ECs provide a structural barrier to prevent the contact between blood-borne pathogens and subendothelial tissues. However, in the pathological processes of atherosclerosis, the excessive production of inflammatory mediators by ECs contributes to disease development [Vita, 2011]. Thus, identifying key regulatory elements controlling the proinflammatory activities of ECs and clarifying their mechanisms of action are of great significance.

Emerging evidences indicated the involvement of long noncoding RNAs (lncRNAs) in regulating the pathological processes of cardiovascular diseases. lncRNAs are long RNAs (>200 nt) without protein-coding ability [Guttman et al., 2009; Rinn and Chang, 2012]. Despite their noncoding nature, lncRNAs can affect gene expression by multiple mechanisms such as chromatin modification or interfering with microRNA (miRNA) expression, through which lncRNAs participate in modulating diverse types of physiological and pathological processes. In recent years, a series of lncRNAs have been identified to be associated with cardiovascular diseases, including atherosclerosis [Zhang et al., 2018]. Among these lncRNAs, metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is highly expressed in vascular endothelium, but its exact functional outcomes in cardiovascular diseases are multifactorial and therefore remain controversial. For example, silencing of MALAT1 expression inhibited the proliferation and regulated the migratory ability of ECs [Michalik et al., 2014]. On the other hand, MALAT1 protected vascular ECs from apoptosis triggered by oxygen-glucose deficiency [Wang et al., 2018]. In macrophages, oxidized low-density lipoprotein (ox-LDL)-induced expression of MALAT1 facilitated the recruitment of β-catenin to the promoter region of *CD36*, thereby augmenting the lipid uptake capacity of macrophages [Rogers et al., 2018]. In contrast, MALAT1 was also reported to promote the activation of the NLRP3 inflammasome and the pyroptosis of macrophages, and thus aggravated inflammatory responses in rats with diabetic atherosclerosis [Han et al., 2018]. Therefore, the detailed influences of MALAT1 on cardiovascular diseases can be either protective or detrimental, probably depending on the type of MALAT1-expressing cells and the kind of stimulus these cells received.

miR-590 is located proximally in the long arm of human chromosome 7 and is an intron-type miRNA [Monteys et al., 2010]. A current study found that miR-590 is closely related to atherosclerotic diseases [Luo et al., 2016]. Shan et al. [2009] showed that miR-590 expression was decreased in cardiovascular disease, suggesting that it may be a protective miRNA. In addition, miR-590 was reported to be involved in the regulation of the inflammatory response [Sheikholeslami et al., 2017]. miR-590 attenuated lipid accumulation and proinflammatory cytokine secretion by targeting the lipoprotein lipase gene in human THP-1 macrophages [He et al., 2014]. In our preliminary study, we found that a miR-590 binding site exists on MALAT1 by Starbase database (http://starbase.sysu.edu.cn/index.php), but their relationship has not been reported yet. So, we speculated that MALAT1 may promote endothelial inflammation by interacting with miR-590. In the present study, we demonstrate that MALAT1 enhances the inflammatory activity while it inhibits nitric oxide (NO) production and cell migratory ability in human umbilical vein ECs (HUVECs) and human coronary artery ECs (HCAECs) via downregulating the expression of miR-590, thereby counteracting its ability to suppress the expression of STAT3, an inflammatory transcription factor. These findings uncover a new role of MALAT1 in regulating endothelium function, and may hopefully be beneficial for understanding the pathological processes of atherosclerosis.

## Material and Methods

*Cell Culture and Treatment*

The human EC lines HCAEC and HUVEC were purchased from Lonza (Basel, Switzerland). Cells were maintained in DMEM/F-12 medium (#11320082, Gibco, USA) containing 10% fetal bovine serum (#16000044, Gibco), heparin (#H3393, Sigma, USA), and EC growth supplement (#354006, BD, Bioscience, USA). All cell lines were routinely maintained at 37°C under 5% $CO_2$ in a humidified incubator. HCAECs and HUVECs in the 3rd to 7th passage were applied in the following experiments. ox-LDL was used to induce a cell inflammation model at the concentration of 50 μg/mL for 24 h.

*Cell Transfection*

The MALAT1 sequence was synthesized and subcloned into plasmid to generate the recombinant vector pcDNA3.1-MALAT1, and the sequence was verified by sequencing. Short hairpin (sh) RNAs directed against human MALAT1 and human STAT3 were constructed in pSicoR vector (shMALAT1 and shSTAT3) (GenePharma, Shanghai, China). Plasmid carrying a non-targeting sequence was used as a negative control (NC). miR-590 mimics, miR-590 inhibitor, and the corresponding control mimics (mimics NC) or control inhibitor (inhibitor NC) were purchased from GenePharma. The sequence of miR-590 mimics was 5′-CAGGCCGAUUGCGAUGCAAUA-3′ and that of miR-590 inhibitor was 5′-AAAUAUGCUGUAUGUCAUGUGUU-3′. HCAECs and HUVECs were seeded into 12-well plates and cultured overnight.

Then, cells under 60–70% confluence were serum starved for 12 h, followed by transfection with pcDNA3.1-MALAT1, shMALAT1, shSTAT3, miR-590 mimics, or miR-590 inhibitor using Lipofectamine 2000 Transfection Reagent (#11668019, Thermo Fisher Scientific, USA) following the manufacturer's protocol. After 48 h transfection, the cells were collected for subsequent experiments.

*Quantitative Real-Time PCR*

Total cell RNA was extracted using Trizolreagent (#345662, Takara, Dalian, China) and was synthesized into cDNA using RevertAid RT Reverse Transcription Kit (#K1691, Thermo Fisher Scientific). Quantitative real-time PCR (qRT-PCR) was conducted on an ABI 7500 using QuantiNova SYBR Green PCR kit (#208052, Qiagen, Hilden, Germany). The amplification conditions were as follows: initial denaturation at 95°C for 10 min, followed by 35 cycles of 10 s at 95°C, 15 s at 60°C, and 10 s at 72°C. Primers used in PCR were MALAT1 forward 5′-AGGTAAAGCTTGAGAAGAT-3′, reverse 5′-GGAAGTAATTCAAGATCAA-3′; miR-590 forward 5′-GAGCTTATTCATAAAAGT-3′, reverse 5′-TCCACGACACGCACTGGATACGAC-3′; STAT3 forward 5′-GCTGCCCCATACCTGAAGAC-3′, reverse 5′-GTAGGCGCCTCAGTCGTATC-3′; U6 forward 5′-CTCGCTTCGGCAGCACA-3′, reverse 5′-AACGCTTCACGAATTTGCGT-3′; and β-actin forward 5′-AAATCTGGCACCACACCTTC-3′, reverse 5′-GGGGTGTTGAAGGTCTCAAA-3′. Data were analyzed using $2^{-\Delta\Delta Ct}$ method. β-actin was used as internal reference for MALAT1 and STAT3, U6 as internal reference for miR-590.

*ELISA Test*

The culture supernatants of HCAECs and HUVECs were collected and subjected to ELISA analysis using TNF-α, IL-6 or IL-8 ELISA kits (#BMS223HS, BMS213HS, and KHC0081, Thermo Fisher Scientific) according to the manufacturer's instructions.

*Wound Healing Assay*

Cells were seeded in 6-well plates, grown to 90% confluence, and scratched with a 200-μL pipette tip at the center of the well. Then, cells were incubated in serum-free medium at 37°C for 24 h. The images of each well were recorded by an inverted microscope (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) at 0 and 24 h. ImageJ software (NIH, Bethesda, MD, USA) was used to calculate the relative distance as follows: $(W_{0h}-W_{24h})/W_{0h} \times 100\%$, where W represents the width of the scratch at the specific time point.

*NO Detection*

The culture medium of HCAECs and HUVECs was collected for NO detection (#S0023, Beyotime, Shanghai, China). The standard solutions were diluted according to the manufacturer's instruction. After adding NADPH, FAD, and nitrate reductase to the 96-well plate, samples and standard solutions were incubated at 37°C for 30 min. Then, LDH Buffer and LDH were mixed for 30 min incubation at 37°C. After incubating with Griess Reagent I and Griess Reagent II for 10 min at room temperature, the 96-well plate was placed into a microplate reader (Thermo Fisher Scientific) for absorbance detection at 540 nm.

*Western Blotting*

The proteins were extracted using RIPA buffer (#P0013C, Beyotime) with protease inhibitors (#04693116001, Roche, Mannheim, Germany). Concentrations of whole-cell lysates were measured using BCA protein quantitation kit (#P0006, Beyotime). Equivalent amounts of protein were separated by 10% SDS-PAGE before being transferred to polyvinylidene difluoride (PVDF) membranes, which were blocked with 5% non-fat milk, incubated with appropriate primary antibodies, and finally probed with HRP-conjugated secondary antibodies. All primary antibodies including anti-GAPDH (#5174), anti-VEGF (#2463), anti-HSP70 (#4873), anti-ICAM1 (#67836), anti-VCAM1 (#39036), anti-TNF-α (#11948), anti-IL-6 (#12912), and anti-IL-8 (#94853) were purchased from Cell Signaling Technology (Danvers, MA, USA). Secondary antibodies included goat anti-rabbit IgG HRP (1:5,000; #ab6721, Abcam, Cambridge, MA, USA) and goat anti-mouse IgG HRP (1:5,000; #ab205719, Abcam). The chemiluminescence signal was visualized following the addition of Immobilon Western Chemiluminescent HRP Substrate (#WBKLS0050, EMD Millipore, Billerica, MA, USA). Quantification of Western blot results was performed using ImageJ software (NIH). GAPDH served as a loading control, and Western blot quantification was normalized to GAPDH.

*Dual-Luciferase Reporter Assay*

We found that the binding site of miR-590 on MALAT1 and STAT3 3′-UTR is AUAAGCU by Starbase database (http://starbase.sysu.edu.cn/index.php). Wild-type or mutant MALAT1 and STAT3 3′-UTR was cloned into psiCHECK-2 luciferase plasmid (Promega, Madison, WI, USA), which was co-transfected with miR-590 mimics into HCAECs and HUVECs using Lipofectamine 2000 Transfection Reagent. Cells were harvested 48 h after transfection and analyzed for luciferase activity using the Luciferase Assay System (Promega, Madison, WI, USA). Firefly luciferase was used for internal control.

*Statistical Analysis*

All experiments were conducted in at least 3 independent replications at 3 times, and data are shown as mean ± SD. Student *t*-test and one-way analysis of variance (ANOVA) with Tukey post-hoc test were used for analysis of 2 groups or multiple group comparison using SPSS19.0 software, respectively. $p < 0.05$ was considered statistically significant.

## Results

### *MALAT1 Is Upregulated in ECs Treated with ox-LDL and MALAT1 Knockdown Inhibits ox-LDL-Induced EC Inflammation*

To corroborate the role of MALAT1 in the inflammatory activity of ECs, ox-LDL was used to stimulate cell inflammatory response. After ox-LDL treatment, the expression level of MALAT1 was significantly increased in HCAECs and HUVECs (Fig. 1A). shMALAT1 transfection reduced the expression level of MALAT1 (Fig. 1B). Furthermore, the concentrations of IL-6, IL-8, and TNF-α were evaluated by ELISA assay (Fig. 1C). In the ox-LDL and ox-LDL + shNC groups, the levels of IL-6,

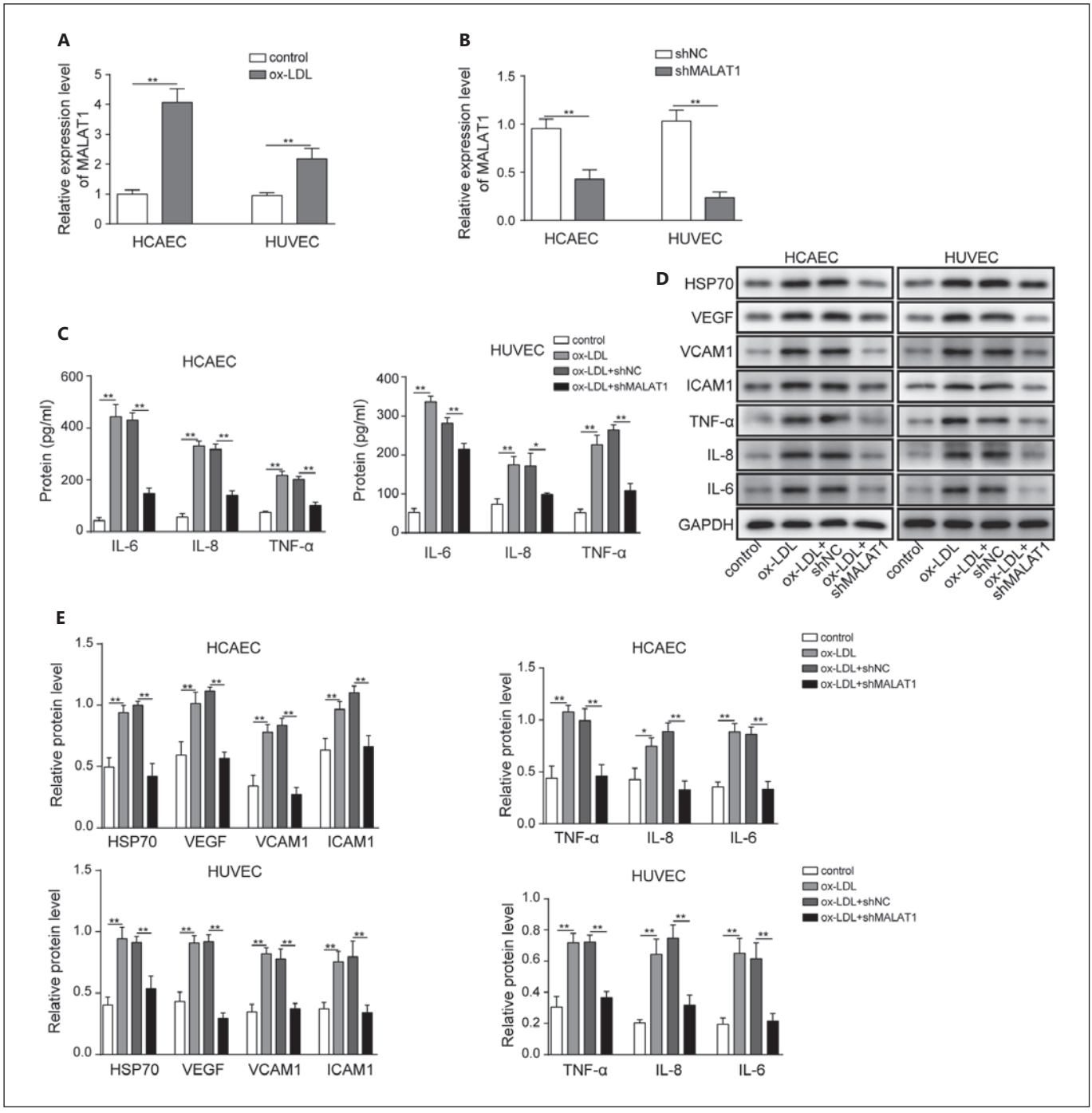


**Fig. 1.** MALAT1 is upregulated in endothelial cells during the pathological process of inflammation. **A**, **B** Expression levels of MALAT1 examined by qRT-PCR in cells treated with ox-LDL (**A**) and in cells transfected with short hairpin RNA directed against MALAT1 (shMALAT1) or a negative control (shNC) (**B**). **C** Concentrations of IL-6, IL-8, and TNF-α in culture supernatant evaluated by ELISA. **D** Protein levels of TNF-α, IL-6, IL-8, ICAM1, VCAM1, HSP70, and VEGF evaluated by Western blotting. GAPDH was set as loading control. **E** Quantification of the results in **D**. Data are shown as means ± SD and are representative of 3 independent experiments. * $p < 0.05$; ** $p < 0.01$.


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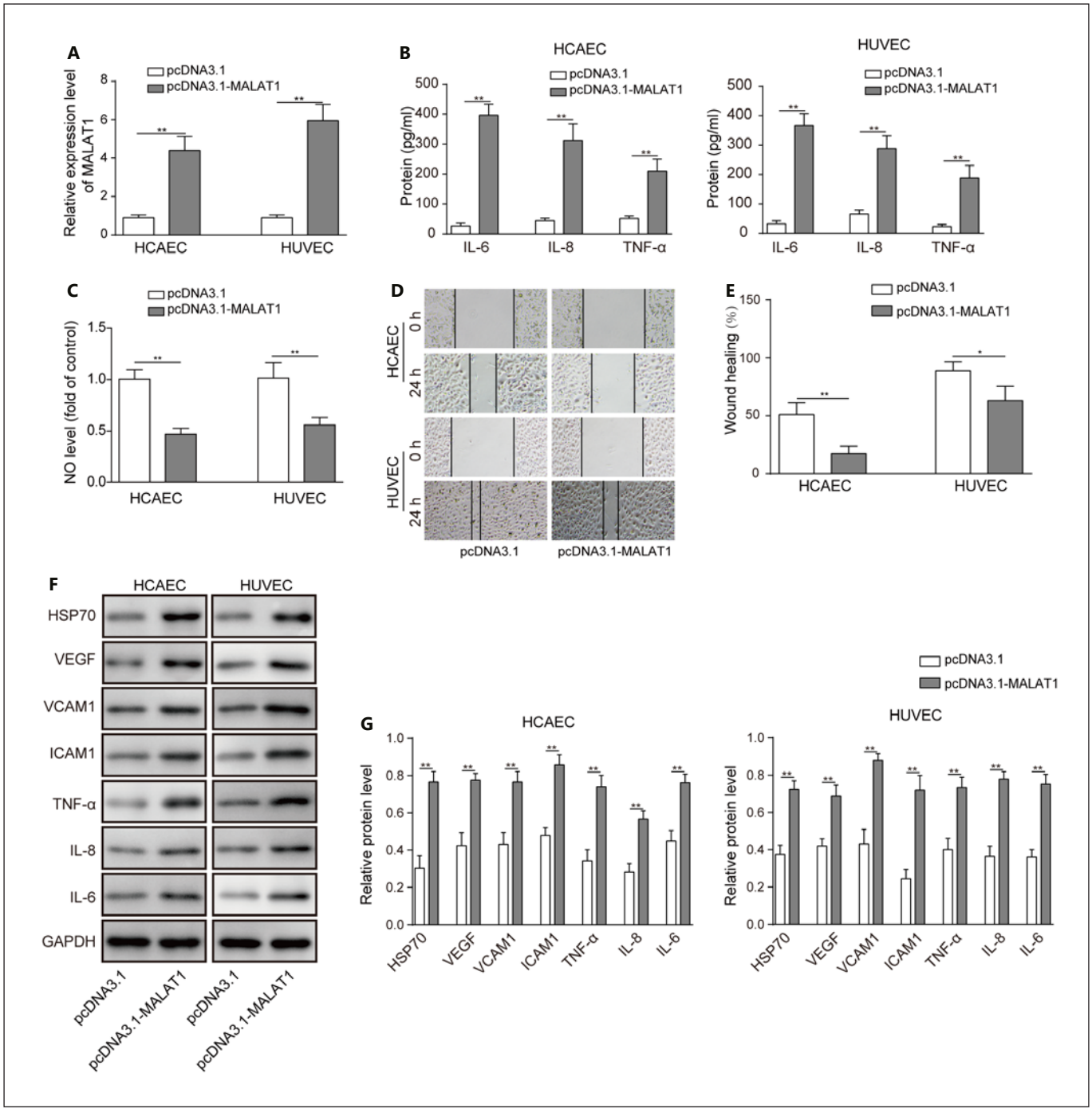


**Fig. 2.** MALAT1 promotes the inflammatory phenotypes of endothelial cells. HCAECs and HUVECs were transfected with pcDNA3.1 or pcDNA3.1-MALAT1 vector. **A** Expression level of MALAT1 examined by qRT-PCR. **B** Concentrations of IL-6, IL-8, and TNF-α in culture supernatant evaluated by ELISA. **C** Level of nitric oxide (NO) in HCAECs and HUVECs. **D** Migratory ability of HCAECs and HUVECs after transfection measured by wound healing assay. **E** Quantification of the results in **D**. **F** Protein levels of TNF-α, IL-6, IL-8, ICAM1, VCAM1, HSP70, and VEGF evaluated by Western blotting. GAPDH was set as loading control. **G** Quantification of the results in **F**. Data are shown as means ± SD and are representative of 3 independent experiments. * $p < 0.05$; ** $p < 0.01$.

IL-8, and TNF-α were greatly increased, which was inhibited in cells with MALAT1 knockdown. Moreover, the protein levels of TNF-α, IL-6, IL-8, ICAM1, VCAM1, HSP70, and VEGF were evaluated by Western blotting (Fig. 1D, E). Similarly, the increased levels of these proteins induced by ox-LDL could be inhibited by the treatment with shMALAT1. Thus, the results demonstrated that ox-LDL can promote the expression of MALAT1 and increase the expression of inflammatory factors and cell adhesion factors in ECs. Knockdown of MALAT1 significantly inhibited ox-LDL-induced inflammation, suggesting that MALAT1 plays a key role in endothelial inflammation.

*MALAT1 Potentiates the Inflammatory Activity in ECs*

In order to verify that the MALAT1/miR-590/STAT3 axis has the same effect on inflammation response of ECs from different sources, we selected 2 ECs, HUVECs and HCAECs, which are commonly used to study the cellular mechanism of EC injury in atherosclerosis [Barroso et al., 2016; Evrard et al., 2016]. We first examined the potential effect of MALAT1 on the proinflammatory activity of ECs. To this end, HCAECs and HUVECs were transfected with empty or MALAT1-expressing plasmid. qRT-PCR demonstrated a successful overexpression of MALAT1 in these cells (Fig. 2A). The ELISA results showed that the levels of TNF-α, IL-6, and IL-8 were higher in the culture supernatant of MALAT1-overexpressing HCAECs and HUVECs than in that of the control cells (Fig. 2B). Furthermore, the NO levels and cell migratory ability were significantly repressed in HCAECs and HUVECs transfected with MALAT1-expressing plasmid (Fig. 2C–E). In addition, overexpression of MALAT1 significantly potentiated the levels of inflammatory cytokine TNF-α, IL-6, and IL-8, as well as the levels of adhesion molecule ICAM1 and VCAM1 (Fig. 2F, G). On the other hand, the expression of VEGF and HSP70 was also increased by MALAT1 overexpression (Fig. 2F, G). Therefore, MALAT1 enhances the inflammatory phenotypes of ECs by supporting their expression of inflammatory cytokines, adhesion molecules, and vascular protective molecules.

*miR-590 Is a Target of MALAT1 and Inhibits Inflammatory Response in ECs*

To mechanistically explore the function of MALAT1 in ECs, we assessed the relationship between miR-590 and MALAT1. Figure 3A shows a schematic diagram of miR-590 and MALAT1 for the luciferase assay. We found that miR-590 was a potential target for MALAT1 3′-UTR through bioinformatics analysis (Fig. 3A). Results of the dual luciferase reporter assay showed that miR-590 significantly downregulated the luciferase activity in ECs expressing wild-type (WT) MALAT1 but not in those expressing mutant (MUT) MALAT1 (Fig. 3B), indicating that miR-590 was a target of MALAT1. Overexpression of MALAT1 or miR-590 inhibitor significantly decreased miR-590 expression in both HCAECs and HUVECs (Fig. 3C). Furthermore, miR-590 inhibition resulted in elevated concentrations of TNF-α, IL-6, and IL-8 in the culture supernatant of HCAECs and HUVECs (Fig. 3D). The NO levels of HCAECs and HUVECs after inhibiting miR-590 were both significantly decreased compared with the inhibitor NC group (Fig. 3E). And the cell migratory ability of HCAECs and HUVECs transfected with miR-590 inhibitor was inhibited (Fig. 3F, G). Next, we analyzed the impact of miR-590 on the inflammatory capacity in ECs. Similar to the effect of MALAT1 overexpression, miR-590 inhibition increased the levels of inflammatory cytokines (TNF-α, IL-6, and IL-8), adhesion molecules (ICAM1 and VCAM1), and VEGF and HSP70 in HCAECs and HUVECs (Fig. 3H, I). These results fully demonstrated that MALAT1 inhibits the expression of miR-590 to promote EC inflammation.

**Fig. 3.** miR-590 is a target of MALAT1 and represses inflammation in endothelial cells. **A** Schematic diagram of miR-590 and MALAT1 3′-UTR for the luciferase assay, and sequence alignment between MALAT1 and miR-590. **B** Luciferase activity in HCAECs and HUVECs co-transfected with miR-590 mimics or mimics NC, and luciferase vectors expressing wild-type or mutant MALAT1. **C** Expression levels of miR-590 in HCAECs and HUVECs transfected with pcDNA3.1, pcDNA3.1-MALAT1, miR-590 inhibitor, or inhibitor NC, quantified by qRT-PCR. **D** Concentrations of IL-6, IL-8, and TNF-α in culture supernatant of HCAECs and HUVECs transfected with miR-590 inhibitor or inhibitor NC, evaluated by ELISA. **E** Level of NO in HCAECs and HUVECs transfected with miR-590 inhibitor or inhibitor NC. **F** Migratory ability of HCAECs and HUVECs after miR-590 inhibitor or inhibitor NC transfection measured by wound healing assay. **G** Quantification of the results in **F**. **H** Protein levels of TNF-α, IL-6, IL-8, ICAM1, VCAM1, HSP70, and VEGF detected by Western blotting in ECs transfected with miR-590 inhibitor or inhibitor NC. GAPDH was set as loading control. **I** Quantification of the results in **H**. Data are shown as means ± SD and are representative of 3 independent experiments. * $p < 0.05$; ** $p < 0.01$.

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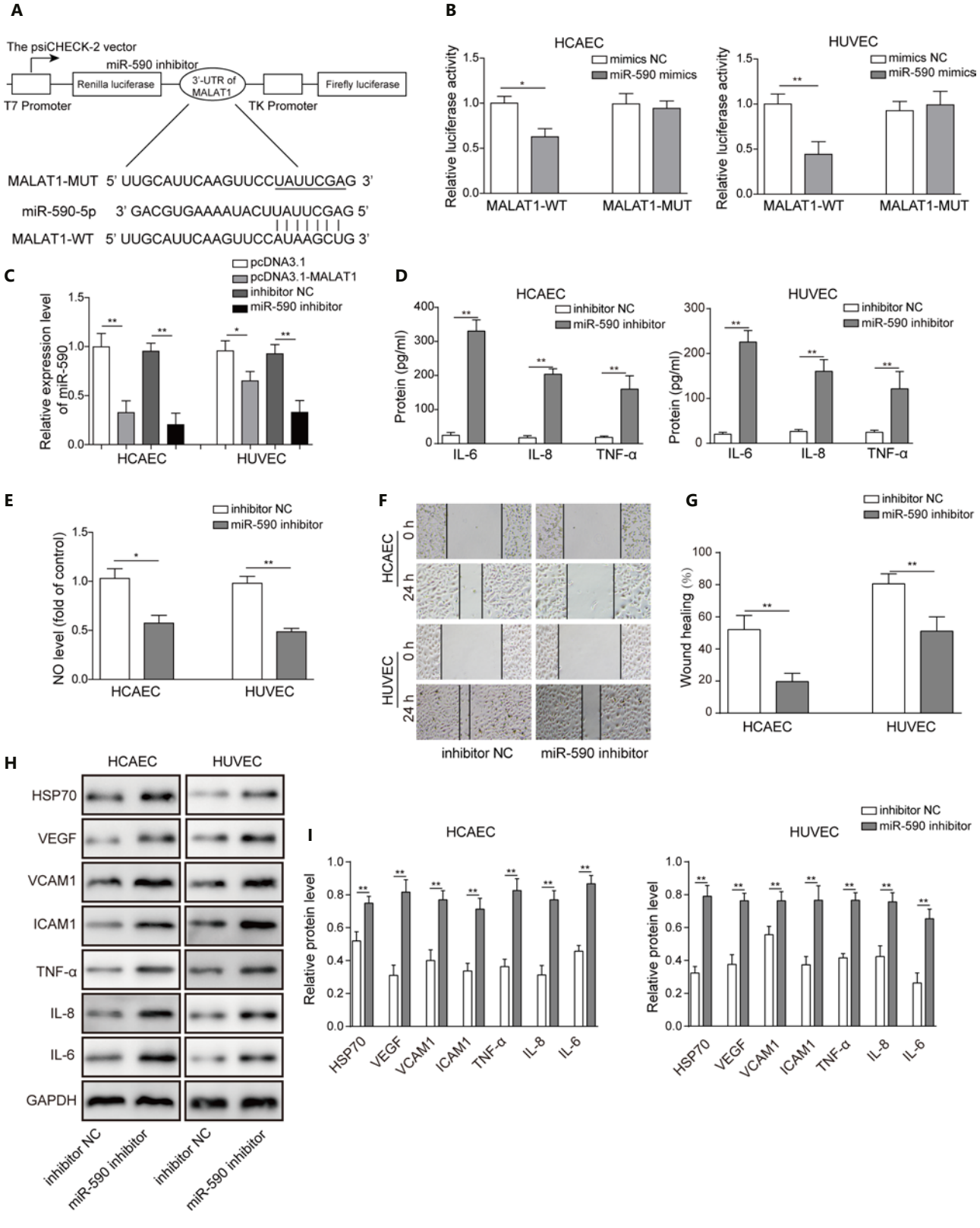


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*miR-590 Suppresses STAT3 Signaling by Binding to the 3′-UTR of STAT3 in ECs*

Furthermore, we examined the relationship between miR-590 and STAT3. Figure 4A shows a schematic diagram of miR-590 and STAT3 for the luciferase assay. We then predicted the target of miR-590 using bioinformatics analysis, and found that the 3′-UTR region of STAT3, an inflammatory transcription factor, contained a seed region recognized by miR-590 (Fig. 4A). The dual luciferase reporter assay showed that the relative luciferase activity was significantly lower in STAT3-WT when miR-590 was overexpressed, and no significant difference was found in STAT3-MUT (Fig. 4B). qRT-PCR demonstrated that miR-590 inhibitor significantly increased the STAT3

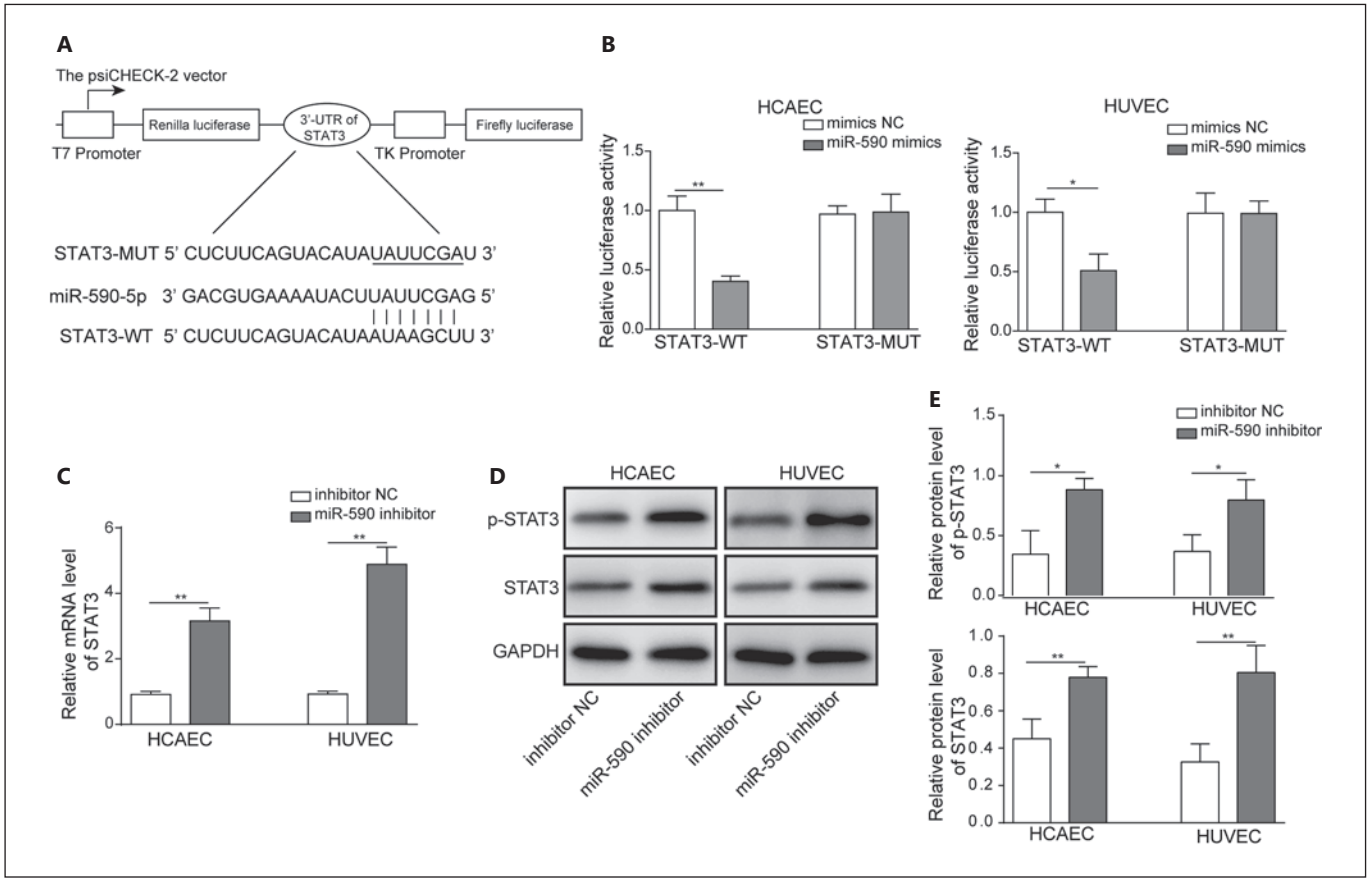


**Fig. 4.** miR-590 directly suppresses STAT3 activity in endothelial cells. **A** Schematic diagram of miR-590 and STAT3 3′-UTR for the luciferase assay, and sequence alignment between miR-590 and STAT3. **B** Luciferase activity in HCAECs and HUVECs co-transfected with miR-590 mimics or mimics NC and luciferase vectors expressing wild-type or mutant STAT3. **C** Expression level of STAT3 quantified by qRT-PCR in HCAECs and HUVECs transfected with miR-590 inhibitor or inhibitor NC. **D** Protein levels of total STAT3 and phosphorylated STAT3 were examined by Western blotting in HCAECs and HUVECs transfected with miR-590 inhibitor or inhibitor NC. GAPDH was set as loading control. **E** Quantification of the results in **D**. Data are shown as means ± SD and are representative of 3 independent experiments. * $p < 0.05$; ** $p < 0.01$.

**Fig. 5.** MALAT1 promotes STAT3-mediated endothelial inflammation in a miR-590-dependent manner. HCAECs and HUVECs were transfected with pcDNA3.1-MALAT1 or miR-590 mimics. **A** mRNA level of STAT3 evaluated by qRT-PCR. **B** Concentrations of IL-6, IL-8, and TNF-α in culture supernatant measured by ELISA. **C** Level of NO in HCAECs and HUVECs. **D** Migratory ability of HCAECs and HUVECs measured by wound healing assay. **E** Quantification of the results in **D**. **F** The protein levels of STAT3, p-STAT3, TNF-α, IL-6, IL-8, ICAM-1, VCAM-1, HSP70, and VEGF were evaluated by Western blotting. GAPDH was set as loading control. **G** Quantification of the results in **F**. Data are shown as means ± SD and are representative of 3 independent experiments. * $p < 0.05$; ** $p < 0.01$.

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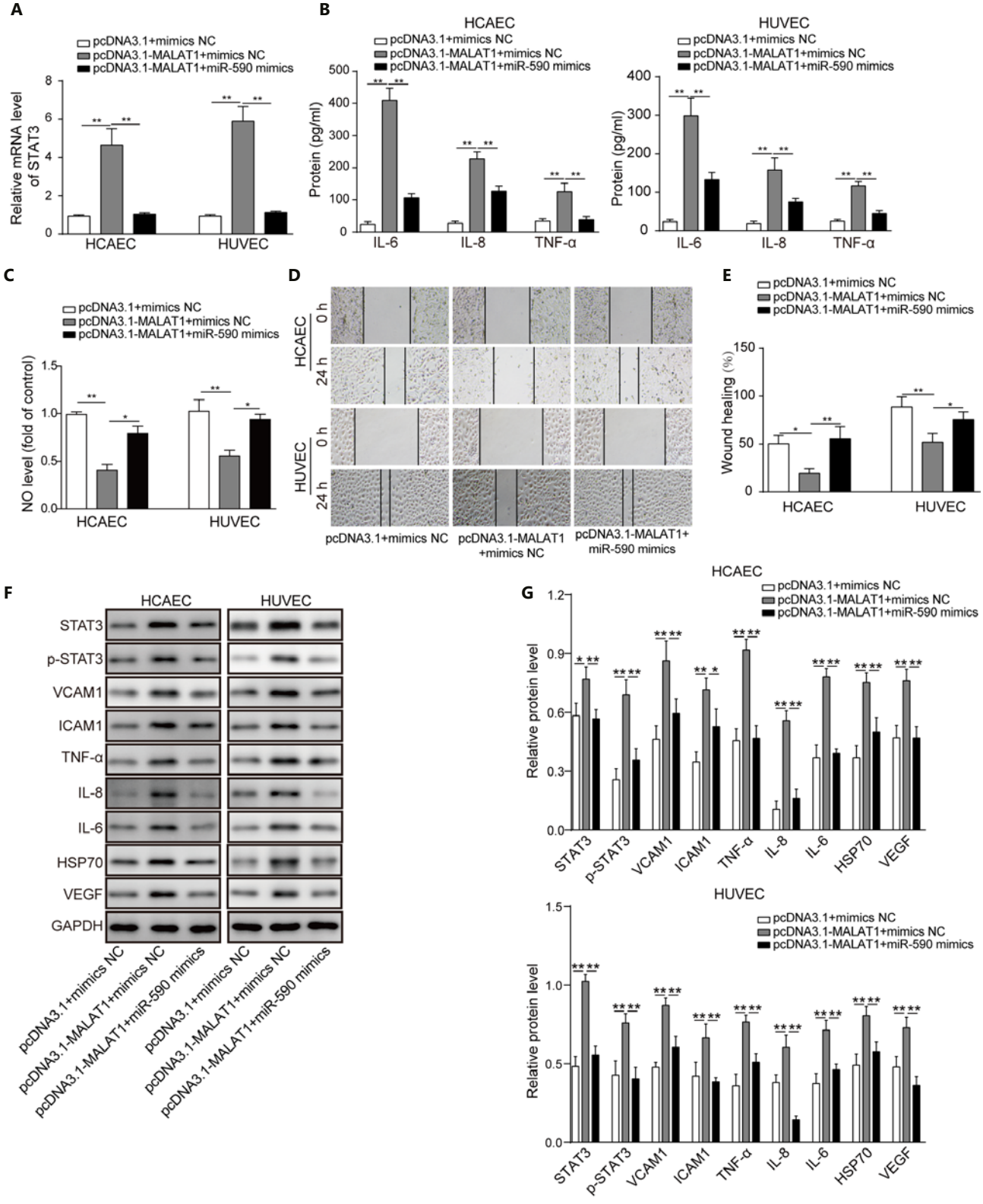


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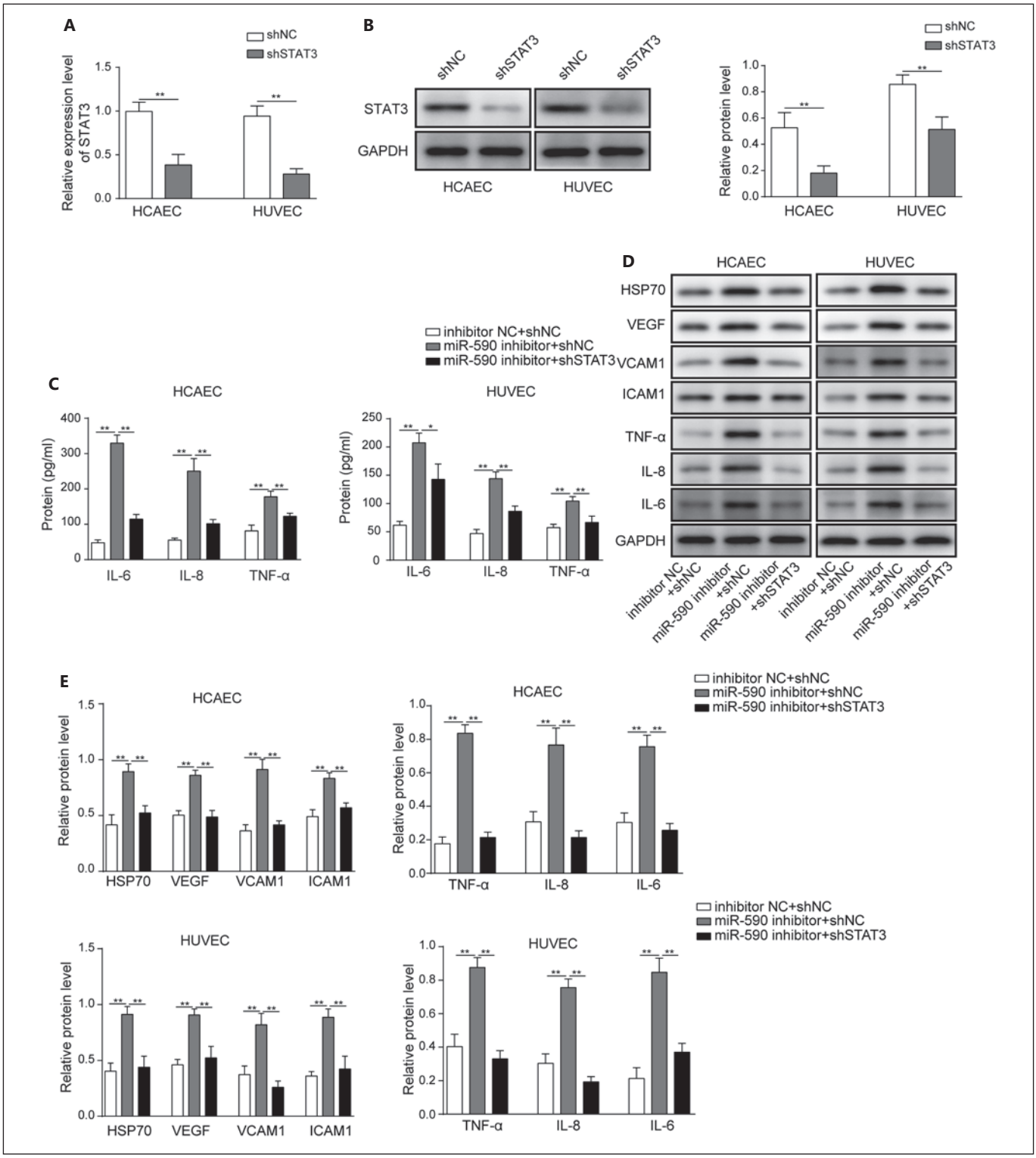


**Fig. 6.** Knockdown of STAT3 reverses the inflammatory response induced by miR-590 inhibition. **A**, **B** mRNA (**A**) and protein (**B**) level of STAT3 examined by qRT-PCR and Western blotting in cells transfected with shSTAT3. **C** Concentrations of IL-6, IL-8, and TNF-α in culture supernatant evaluated by ELISA. **D** Protein levels of TNF-α, IL-6, IL-8, ICAM1, VCAM1, HSP70, and VEGF evaluated by Western blotting. GAPDH was set as loading control. **E** Quantification of the results in **D**. Data are shown as means ± SD and are representative of 3 independent experiments. * $p < 0.05$; ** $p < 0.01$.

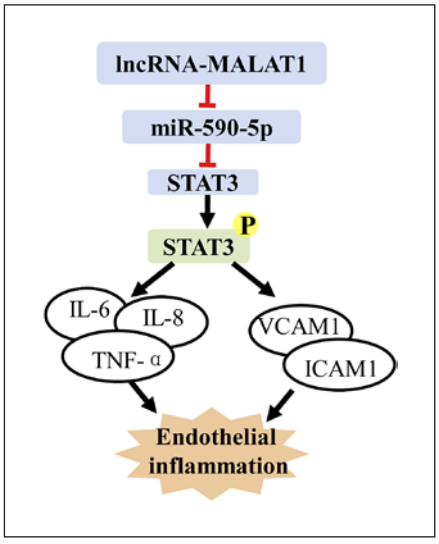


**Fig. 7.** Diagram summarizing the MALAT1/miR-590/STAT3 axis in endothelial cells. MALAT1 could activate the STAT3 signaling pathway by targeting miR-590, thereby promoting the inflammatory response of vascular endothelial cells.

expression level (Fig. 4C). At protein level, total STAT3 and phosphorylated STAT3 were also enhanced by miR-590 inhibitor (Fig. 4D, E). These findings suggested that miR-590 inhibits STAT3 activity by binding to the 3′-UTR of STAT3 in ECs.

*MALAT1 Regulates STAT3 Activity and Endothelial Inflammation in a miR-590-Dependent Manner*

To further verify the MALAT1/miR-590/STAT3 regulatory axis, pcDNA3.1-MALAT1 and miR-590 mimics were co-transfected to HCAECs and HUVECs. Overexpression of MALAT1 significantly enhanced expression of STAT3, while miR-590 mimics reversed this change (Fig. 5A). The increased secretion of inflammatory cytokines (IL-6, IL-8, and TNF-α) in MALAT1-overexpressing ECs was also offset by the concomitant overexpression of miR-590 (Fig. 5B). Overexpression of MALAT1 in HCAECs and HUVECs decreased the NO level, which was increased to the control level in HCAECs and HUVECs co-transfected with pcDNA3.1-MALAT1 and miR-590 mimics (Fig. 5C). The cell migratory ability was inhibited in the ECs transfected with pcDNA3.1-MALAT1, and overexpression of miR-590 in HCAECs and HUVECs could reverse the above effect caused by overexpression of MALAT1 (Fig. 5D, E). Furthermore, MALAT1 significantly promoted the phosphorylation of STAT3, and increased the expression of IL-6, IL-8, TNF-α, ICAM1, VCAM1, HSP70 and VEGF, whereas these effects of MALAT1 were reversed by miR-590 mimics (Fig. 5F, G). Collectively, MALAT1 promoted the STAT3-mediated inflammatory activity in ECs through directly downregulating miR-590.

*Knockdown of STAT3 Reverses the Inflammatory Response Induced by miR-590 Inhibition*

In order to prove the role of STAT3 on miR-590 in mediating the endothelial inflammation, shSTAT3 was constructed and transfected into cells. After transfection, the mRNA and protein level of STAT3 were significantly reduced in HCAECs and HUVECs (Fig. 6A, B), proving that shSTAT3 transfection was successful. The concentrations of IL-6, IL-8, and TNF-α were detected by ELISA assay (Fig. 6C). Knockdown of miR-590 dramatically inhibited the levels of IL-6, IL-8, and TNF-α in HCAECs and HUVECs, while after transfecting with shSTAT3, the activated inflammatory response was obviously inhibited. Also, the increased protein levels of TNF-α, IL-6, IL-8, ICAM1, VCAM1, HSP70, and VEGF caused by transfecting miR-590 inhibitor were reduced after knocking down of STAT3 (Fig. 6D, E). The above results indicated that miR-590 knockdown activates the inflammatory response, which was reversed by STAT3 inhibition. In Figure 7, a diagram summarizing the MALAT1/miR-590/STAT3 axis in ECs is depicted. It demonstrates that MALAT1 activates the STAT3 signaling pathway by targeting miR-590, thereby promoting the inflammatory response.

## Discussion

Atherosclerosis is the major cause of vascular disorders which causes approximately 30% of all deaths worldwide [Brundtland, 2002]. It is well-accepted that the development of atherosclerosis is closely associated with the inflammatory status of vascular ECs. Elevated production of inflammatory cytokines (such as TNF-α and IL-6) or increased expression of adhesion molecules (such as ICAM1 and VCAM1) were reported to aggravate pathological processes of atherosclerosis [Blankenberg et al., 2003; Schuett et al., 2009; Kleinbongard et al., 2010]. Identifying key molecular elements regulating the inflammatory phenotypes of vascular ECs is not only helpful for understanding the pathology of atherosclerosis, but also can provide candidate targets for the diagnosis or treatment of this disease.

MALAT1 was initially discovered as a tumor-associated lncRNA that controls proliferation and metastasis [Ji et al., 2003; Dhamija and Diederichs, 2016]. Previous studies showed that MALAT1 is closely related to the occurrence of cardiovascular disease, including atherosclerosis [Gao et al., 2019; Hu et al., 2019]. It was reported that MALAT1 aggravated cardiac inflammation by interaction with miR-125b and p38 MAPK/NF-κB [Chen et al., 2018]. Knockdown of MALAT1 could inhibit the expression of TNF-α and IL-6 in HUVECs, suggesting that MALAT1 promotes EC inflammation [Puthanveetil et al., 2015]. Furthermore, exosomal MALAT1 derived from HUVECs promoted inflammatory response in atherosclerotic mice [Gao et al., 2019]. Our study demonstrates that MALAT1 potentiates the inflammatory activity and inhibits cell migration in ECs, which is consistent with the previous report. However, a recent study suggested that knockout of MALAT1 promoted atherosclerotic lesion formation in mice and elevated levels of proinflammatory mediators [Cremer et al., 2019]. We suspected that the possible cause was that MALAT1 participates in the regulation of various biological processes in different cells. Knockout of MALAT1 in mice may regulate other cell function signals in addition to affecting ECs, thereby causing changes in the levels of inflammatory mediators. The effect of MALAT1 on atherosclerosis is still controversial and needs further study.

STAT3 is a well-characterized inflammatory transcription factor which can be activated by a wide range of extracellular or intracellular stimuli, including IL-6 family members IL-6, IL-11, IL-27, leukemia inhibitory factor, IL-10 family members IL-10, IL-22, epidermal growth factor, VEGF, or environmental stimuli such as hypoxia or glucose deprivation [Carlsson et al., 2018; Fang et al., 2018]. Many evidences have indicated that overactivation of STAT3 signaling is associated with the initiation or progression of endothelial inflammation. For instance, in human vascular ECs, JAK/STAT3 signaling activated by IL-6 is a major contributor to the development of endothelial inflammation [Zegeye et al., 2018]. It was also reported that IL-6-induced activation of STAT3 signaling led to the de novo synthesis of a series of proteins that caused endothelial barrier function loss [Alsaffar et al., 2018]. Besides, STAT3 could be activated by IL-22, resulting in endothelial dysfunction and blood pressure elevation in angiotensin II-treated mice [Ye et al., 2017]. Acyloxynitroso compounds could also promote the progression of endothelial and cardiac inflammation through inducing STAT3 phosphorylation [Zgheib et al., 2012]. Therefore, STAT3 is a promising target for the treatment of vascular disease [Dutzmann et al., 2015]. Despite the important effects of STAT3 on endothelial inflammation, few noncoding RNAs have been identified to directly target STAT3 in vascular ECs, and their functional relevance to endothelial inflammation is also far from being understood. In our study, STAT3 was identified to be positively regulated by MALAT1, whose overexpression led to significantly increased production of inflammatory mediators, and the STAT3-inducing role of MALAT1 was dependent on its ability to downregulate miR-590 expression.

miRNAs are short (typically 20–23 bp), noncoding RNA molecules which exert crucial roles in regulating a wide range of physiological or pathological processes. For example, atypical mechanosensitive miR-712 derived from preribosomal RNA exacerbates atherosclerosis by promoting endothelial inflammation [Son et al., 2013]. Suppression of miR-92a expression reduces endothelial inflammation and atherosclerosis in vivo [Loyer et al., 2014]. Previous studies have indicated the oncogenic roles of miR-590 in many kinds of tumors. For example, miR-590 was found to target and downregulate the expression of FasL in acute myeloid leukemia cells, thereby promoting tumor development [Favreau et al., 2013]. Moreover, miR-590 enhanced the growth of hepatocellular carcinoma cells through reducing the expression of TGF-β RII [Jiang et al., 2012]. In addition, miR-590 increased both the proliferation and invasion of clear cell renal carcinoma cells by suppressing PBRM1 expression [Xiao et al., 2012]. Beyond its functions in cancer development, miR-590 was also involved in regulating the pathological processes of Alzheimer disease [Sørensen et al., 2014], metabolic disease [He et al., 2014], and immune disorder [Long et al., 2016]. Nevertheless, whether and how miR-590 participates in the regulation of endothelial inflammation is still unknown. In this study, miR-590 was found to serve as an anti-inflammatory miRNA by downregulating STAT3 expression. In the study of Shen et al. [2016], miR-590 promoted gastric cancer cell growth and inhibited chemosensitivity by regulating AKT/ERK and STAT3 signaling pathways. Furthermore, it was reported that miR-590 repressed proliferation of human fetal airway smooth muscle cells by negatively regulating the expression level of STAT3 and its downstream genes including cyclin D3 and p27 [Shi et al., 2018], which is consistent with our results. *STAT3* is a target gene of miR-590, and miR-590 exerts different biological functions through targeting STAT3. By constructing shSTAT3, we found out that STAT3 inhibition could reverse the inflammation response caused by miR-590 knockdown.

## Conclusions

Our study reveals that MALAT1 serves as a sponge lncRNA for miR-590 and antagonizes its capacity to downregulate the expression of STAT3, thus promoting the development of endothelial inflammation. However, the MALAT1/miR-590/STAT3 axis confirmed in our research has not been verified in an animal model in vivo yet, necessitating further studies. The MALAT1/miR-590/STAT3 regulatory network might become a candidate therapeutic strategy for the treatment of endothelial inflammation-mediated atherosclerosis.

## Statement of Ethics

Ethical approval is not required for this type of research.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.


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## Author Contributions

Q.Z.: Study conception and design, definition of intellectual content, guarantor of integrity of the entire study, literature research, data acquisition, experimental studies, manuscript preparation and editing. Q.R.: Clinical studies, data acquisition. C.-Y.L.: Data analysis. X.-L.W.: Study design, manuscript review. X.-Y.X: Statistical analysis.

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